

# DHA supplementation improved both memory and reaction time in healthy young adults: a randomized controlled trial<sup>1-3</sup>

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## ABSTRACT

**Background:** Docosahexaenoic acid (DHA) is important for brain function, and its status is dependent on dietary intakes. Therefore, individuals who consume diets low in omega-3 (n-3) polyunsaturated fatty acids may cognitively benefit from DHA supplementation. Sex and apolipoprotein E genotype (*APOE*) affect cognition and may modulate the response to DHA supplementation.

**Objectives:** We investigated whether a DHA supplement improves cognitive performance in healthy young adults and whether sex and *APOE* modulate the response.

**Design:** Healthy adults ( $n = 176$ ; age range: 18–45 y; nonsmoking and with a low intake of DHA) completed a 6-mo randomized, placebo-controlled, double-blind intervention in which they consumed 1.16 g DHA/d or a placebo. Cognitive performance was assessed by using a computerized cognitive test battery. For all tests,  $z$  scores were calculated and clustered into cognitive domains as follows: episodic and working memory, attention, reaction time (RT) of episodic and working memory, and attention and processing speed. ANCOVA was conducted with sex and *APOE* as independent variables.

**Results:** RTs of episodic and working memory improved with DHA compared with placebo [mean difference (95% CI):  $-0.18$  SD ( $-0.33, -0.03$  SD) ( $P = 0.02$ ) and  $-0.36$  SD ( $-0.58, -0.14$  SD) ( $P = 0.002$ ), respectively]. Sex  $\times$  treatment interactions occurred for episodic memory ( $P = 0.006$ ) and the RT of working memory ( $P = 0.03$ ). Compared with the placebo, DHA improved episodic memory in women [ $0.28$  SD ( $0.08, 0.48$  SD);  $P = 0.006$ ] and RTs of working memory in men [ $-0.60$  SD ( $-0.95, -0.25$  SD);  $P = 0.001$ ]. *APOE* did not affect cognitive function, but there were some indications of *APOE*  $\times$  sex  $\times$  treatment interactions.

**Conclusions:** DHA supplementation improved memory and the RT of memory in healthy, young adults whose habitual diets were low in DHA. The response was modulated by sex. This trial was registered at the New Zealand Clinical Trials Registry (<http://www.anzctr.org.au/default.aspx>) as ACTRN12610000212055. *Am J Clin Nutr* 2013;97:1134–43.

## INTRODUCTION

The long-chain (LC)<sup>4</sup> omega (n-3) PUFA DHA is the dominant n-3 PUFA in the brain. DHA performs structural functions and influences numerous neuronal and glial cell processes (1–3). DHA has been shown to accumulate in areas of the brain involved in memory and attention such as the cerebral cortex and hippocampus (4, 5), and animal studies have shown that deficiency in brain DHA has critical effects on neuronal development and be-

havior, including changes in learning, memory, auditory, and olfactory responses (3). Despite DHA's critical role in brain function, the capacity to synthesize DHA de novo is limited in mammals, and its consumption through the diet ensures an adequate supply for neuronal function (1). A large proportion of the New Zealand population [ $\sim 30\%$  (6)] do not, or rarely, consume seafood (ie, the major dietary source of DHA), which could potentially affect cognitive function. Research on the efficacy of DHA, in terms of its effects on cognitive function in humans, by using randomized controlled trials (RCTs) has focused on either end of the life cycle and shown improvements in children with learning disorders (7, 8) and inconsistent effects in older adults during age-related cognitive decline (9–11). There is a lack of robust evidence to assess the effect of DHA supplementation on cognitive performance in younger healthy adults; to our knowledge, only 5 trials have been conducted, each with considerable design limitations, including small sample sizes (12–14), short durations (12–16), and a lack of placebo control (13).

Although the physiologic basis is poorly understood, significant sex differences with regard to cognitive performance have been shown (17, 18). Event-related potential and fMRI studies have shown sex differences in the pattern of brain activation (19, 20). Therefore, it is likely that the response in cognitive performance to DHA supplementation may be modulated by sex. However, to our knowledge, none of the RCTs that investigated the effect of DHA supplementation on cognitive function has examined sex interactions.

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<sup>4</sup> Abbreviations used: AA, arachidonic acid; CRT, choice reaction time; LC, long chain; RCT, randomized controlled trial; RT, reaction time.

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Apolipoprotein E genotype (*APOE*) is a major genetic risk factor for Alzheimer's disease with carriers of the *APOE4* allelic variant (~25% of whites) at several-fold increased risk (21, 22). Although the evidence is controversial, it is likely that the *APOE4* allele also affects cognitive performance in cognitively healthy adults (23). Some studies showed poorer performance on cognitive tasks in healthy adult *APOE4* carriers (23), whereas other studies showed no difference (24) or even better performance (25, 26) compared with *APOE4* noncarriers. Structural and functional neurologic changes are seen in *APOE4* carriers decades before the appearance of any cognitive or clinical symptoms (27–30). Prospective and some intervention studies have shown *APOE*-modulating effects on the relation between LC n–3 PUFAs and cognitive function, although the results have been controversial (10, 31–34).

The primary aim of the study was to investigate whether a high-DHA supplement for 6 mo would improve memory (episodic and working memory), attention, reaction times (RTs) of memory and attention, and processing speed in young, healthy adults (age range: 18–45 y) whose habitual diets were low in DHA. A secondary aim was to investigate whether sex and *APOE* would modulate the response to the intervention.

## SUBJECTS AND METHODS

This dietary intervention (<http://www.anzctr.org.au;ACTRN12610000212055>) was conducted at Massey University's Albany campus (New Zealand) between March and December 2010 according to the guidelines of the Declaration of Helsinki. Ethical approval for the trial was obtained from the Massey University Human Ethics Committee (Southern A; reference 10/07), and written informed consent was obtained from all participants.

### Participants

A total of 228 adults (83 men and 145 women), aged 18–45 y, were recruited in Auckland, New Zealand. Inclusion criteria for participants were no known major medical condition or disease and not taking medication for any condition or disease, non-smoking, low habitual intake of LC n–3 PUFAs (less than ~200 mg EPA + DHA/wk), no consumption of fish-oil supplements over the past 6 mo, no allergies to seafood, and not pregnant or lactating. LC n–3 PUFA intake was estimated by asking potential participants to record the frequency of habitual consumption of seafood. Seafood was categorized into fatty (greater than ~1 g n–3/100 g), medium-fat (~0.5–1 g n–3/100g), and low-fat (less than ~0.5 g n–3/100 g) sources (35), and frequency options included never, 1, 2, or 3 times/mo, and 1, 2, or >2×/wk.

### Study design

A randomized, placebo-controlled, double-blind study design was used. Volunteers who met eligibility criteria were randomly assigned to one of 2 groups (ie, the DHA or placebo groups) for a period of 6 mo. The random allocation was done by stratified random assignment on the basis of sex and age. The randomization scheme was generated by using the website Randomization.com (<http://www.randomization.com>). DHA and placebo capsules were supplied by Efamol Ltd and Health & Herbs International Ltd. Treatment was provided as three 750-mg cap-

sules/d. DHA capsules provided 1.16 g DHA/d and 0.17 g EPA/d, and placebo capsules contained high-oleic acid sunflower oil. Fatty acid profiles of treatments are summarized in **Table 1**. The dosage of DHA was chosen to be physiologically relevant and achievable through diet (equivalent to ~2–3 portions oily fish/wk), and the duration of 6 mo was chosen to ensure saturation of the tissues with DHA. Erythrocyte DHA levels which have been shown to correlate with brain tissue levels (36), reach a plateau after 6 mo (37). Placebo and treatment capsules were identical in size and shape. Capsules were provided in identical opaque drug containers that were coded and distributed by staff from Health & Herbs International Ltd according to the randomization scheme. Both research staff and participants were blind as to which participants received DHA or placebo treatments until after data analysis. Participants were requested to consume capsules with a meal and to store capsules in the fridge.

General demographic information, including ethnicity, level of education, and first language, was obtained by using a structured questionnaire. Cognitive assessments, fasting blood samples, and anthropometric measurements were obtained at baseline and after 6 mo. Participants were requested not to consume any fatty fish or fish-oil supplements (other than those provided) and to maintain their normal daily routine (eating pattern, physical activity, and alcohol consumption) for the duration of the study. Participants kept weekly diaries to record the consumption of DHA or placebo capsules, seafood (type and amount), and any deviations from the study protocol (eg, illness and use of medication or other nutritional supplements). The average weekly consumption of seafood portions was calculated from diaries and categorized into fatty, medium-fat, and low-fat sources (as previously described). Treatment compliance was determined by using a combination of weekly diary records, pill-counting of leftover capsules, and analysis of erythrocyte LC n–3 PUFA levels which is a valid biomarker for the intake of LC n–3 PUFA (37).

At the end of the study, each participant completed a computer-based tolerance questionnaire adapted from Freeman and Sinha (38).

### Blood sample collection and assays

EDTA blood was collected. The EDTA buffy coat (white blood cell-rich layer) was used for the extraction of DNA for *APOE* analysis. Erythrocytes were washed 3 times with saline (0.9% NaCl) for fatty acid analysis. Analysis of *APOE* were carried out by Canterbury Health Laboratories, which is fully

**TABLE 1**

Fatty acid composition (g/2.25-g daily dosage) of DHA and placebo capsules<sup>1</sup>

Fatty acids	DHA capsules	Placebo capsules
Palmitic acid (16:0)	0.02	0.09
Stearic acid (18:0)	0.07	0.06
Oleic acid (18:1n–9)	0.13	1.61
Linoleic acid (18:2n–6)	0.02	0.12
Arachidonic acid (20:4n–6)	0.05	<0.01
EPA (20:5n–3)	0.17	0.02
DPA <sup>2</sup> (22:5n–3)	0.06	<0.01
DHA (22:6n–3)	1.16	0.02

<sup>1</sup> Only fatty acids that were detected at ≥1% of total fatty acids for either active or placebo capsules are shown.

<sup>2</sup> DPA, docosapentaenoic acid.

accredited with International Accreditation New Zealand to International Organization for Standardization 15189, by using polymerase chain reaction as described by Hixson and Vernier (39). Erythrocyte fatty acids were analyzed by using a Shimadzu gas chromatograph 2010 ported to a gas chromatograph mass spectrometer-QT2010 (Shimadzu Corp) as previously described (40). Briefly, a 200- $\mu$ L solution of 83  $\mu$ mol/L heptadecanoic acid 17:0 ( $\Sigma > 98\%$ ) as an internal standard that contained butylated hydroxytoluene (150  $\mu$ mol/L) dissolved in methanol was added to 50  $\mu$ L erythrocytes followed by 1 mL methanolic HCL (3N). Samples were vortexed and incubated for 4 h at 90°C. After cooling to room temperature, fatty acid methyl esters were extracted by adding 2 mL hexane followed by thorough mixing. The hexane phase that contained fatty acid methyl esters was recovered after centrifugation, dried under nitrogen, resuspended with 100  $\mu$ L hexane, and transferred to a gas chromatography vial of which 1  $\mu$ L was injected onto the gas chromatography-mass spectrometer via split injection (split ratio of 1:10). The capillary column used was an Rtx-2330 (30 m  $\times$  0.25 mm; Restek). Mass spectrometer conditions were as follows: ion source temperature, 235°C; interface temperature, 250°C; and ionization voltage, 70 eV. The injector temperature was 250°C. Helium gas was used as the carrier gas at a flow rate of 0.77 mL/min. An initial oven temperature of 70°C was maintained for 3.0 min, allowed to increase to 155°C at a rate of 25°C/min, held for 6.0 min, increased to 175°C at a rate of 3°C/min, held for 3.0 min, increased to 205°C at a rate of 3°C/min, and finally increased to 220°C at a rate of 8°C/min and held for 2.0 min. The total time for each run was 36 min. The total spectrum of erythrocyte fatty acids in the samples were identified and quantified and are expressed as the weight percentage of total fatty acids. CVs for EPA and DHA assays were 2.7% and 3.6%, respectively. The CV for the DHA assay was 4%.

### Analysis of supplements

Oxidation levels of supplements during the 6-mo duration of the study were analyzed by measuring the peroxide and anisidine values by using the American Oil Chemists' Society Official Method Cd8-53 with modifications and the American Oil Chemists' Society Official Method Cd18-90, respectively (41). Oxidation levels were below maximum permitted levels. The fatty acid content of the capsules were analyzed by using a Shimadzu GC-17A gas chromatograph equipped with a flame-ionization detector as previously described (42).

### Cognitive assessment

Cognitive function was assessed with the Computerized Mental Performance Assessment System (Northumbria University), which has previously been shown to be sensitive to nutritional interventions (43, 44). The following 2 additional tasks were included; the finding As task from the Kit of Factor-Referenced Cognitive Tests (45) and the letter-number sequencing task, which is a subtest of the Wechsler Adult Intelligence Scale III Intelligence test (46). Cognitive tasks used were all standard tasks of cognitive function that have previously been shown to increase activation of the frontal cortex (15), which is the area of the brain associated with the accumulation of DHA, memory, and attention (4, 5). The battery of tests took  $\sim 1$  h to administer. The following

cognitive domains and tasks were assessed: episodic memory (immediate and delayed word recall, delayed word recognition, and delayed picture recognition), working memory (*n*-back, Corsi blocks, and a letter-number sequencing task), attention [Stroop test, choice reaction time (CRT), and digit vigilance]; and processing speed (finding As task).

The accuracy (percentage of correct responses made) for all tests and RTs (in ms) (only for *n*-back, word and picture recognition, Stroop, CRT, and digit vigilance) were assessed. See online supplementary material under "Supplemental data" in the online issue for detailed descriptions of tasks.

The cognitive testing was conducted under rigorously controlled conditions (see online supplementary material under "Supplemental data" in the online issue). In brief, assessments at baseline and end were performed at a similar time of day (between 0700 and 1000). On the day before tests, participants were instructed not to consume alcohol, take recreational drugs, or undertake any unusual sporting activities, to have a good night's sleep, and not be overly stressed. These aspects were confirmed before participants were allowed to commence assessments. Participants arrived at the research unit fasted from food or stimulants (caffeine and alcohol), except for water, for  $\geq 10$  h. A standard breakfast was provided before the commencement of cognitive tests. Environmental factors such as noise and temperature were controlled to avoid any distraction during tests. All assessments were carried out on 5 standardized computers, with the same computer used by individual participants at baseline and end assessments. Participants were instructed on the procedure for the administration of the cognitive battery and undertook a training session before administration of the full battery of tests at baseline and end assessments.

### Statistical analysis

The sample-size calculation was based on a difference in *z* score of 0.5 for memory domains and proved a statistical power equal to 0.8 and an  $\alpha$  level of 0.05 (2 tailed) (G\*Power 3.1.2) (47). The minimum sample size required was 32 participants per treatment and sex group. To test for sex  $\times$  treatment and *APOE*  $\times$  treatment interactions, a total sample size of 179 provided 80% power to detect a medium effect size *f* of 0.25 (equivalent to a treatment effect of 0.5 SD) at an  $\alpha$  level of 0.05 (G\*Power 3.1.2) (47).

Descriptive and comparison statistics (independent *t* test, 2 tailed) of baseline characteristics were based on all participants randomly assigned to treatment groups. Baseline characteristics of dropouts and participants who completed the study were compared, and dropouts did not differ from study completers.

The primary analysis was carried out on all participants for whom baseline and end data were available irrespective of the level of compliance or protocol violations. The analysis was carried out on 7 cognitive domains, including sex (men compared with women) and *APOE* (*APOE4* carriers compared with noncarriers) as independent variables (28 tests). Changes to cognitive domains during the treatment period between DHA and placebo groups were assessed by using ANCOVA models to adjust for baseline cognitive-function test scores and other covariates as follows: education, first language (English compared with other), age, and baseline DHA concentrations. Sex and *APOE* were added to the model as independent variables to test



for sex  $\times$  treatment,  $APOE \times$  treatment, and  $APOE \times$  sex  $\times$  treatment interactions.

For all cognitive outcomes,  $z$  scores were calculated by pooling baseline and 6-mo data as previously described (9).  $z$  scores were clustered into cognitive domains as follows:

$$\text{Memory} = (z_{\text{immediate word recall}} + z_{\text{delayed word recall}} + z_{\text{delayed word recognition}} + z_{\text{delayed picture recognition}}) \div 4 \quad (1)$$

$$\text{Working memory} = (z_{n\text{-back}} + z_{\text{Corsi blocks}} + z_{\text{letter-number sequencing task}}) \div 3 \quad (2)$$

$$\text{Attention} = (z_{\text{Stroop test}} + z_{\text{CRT}} + z_{\text{digit vigilance}}) \div 3 \quad (3)$$

$$\text{RT of memory} = (z_{\text{RT delayed-word recognition}} + z_{\text{RT delayed-picture recognition}}) \div 2 \quad (4)$$

$$\text{RT of working memory} = z_{\text{RT } n\text{-back}} \quad (5)$$

$$\text{RT of attention} = (z_{\text{RT Stroop test}} + z_{\text{RT choice RT}} + z_{\text{RT digit vigilance}}) \div 3 \quad (6)$$

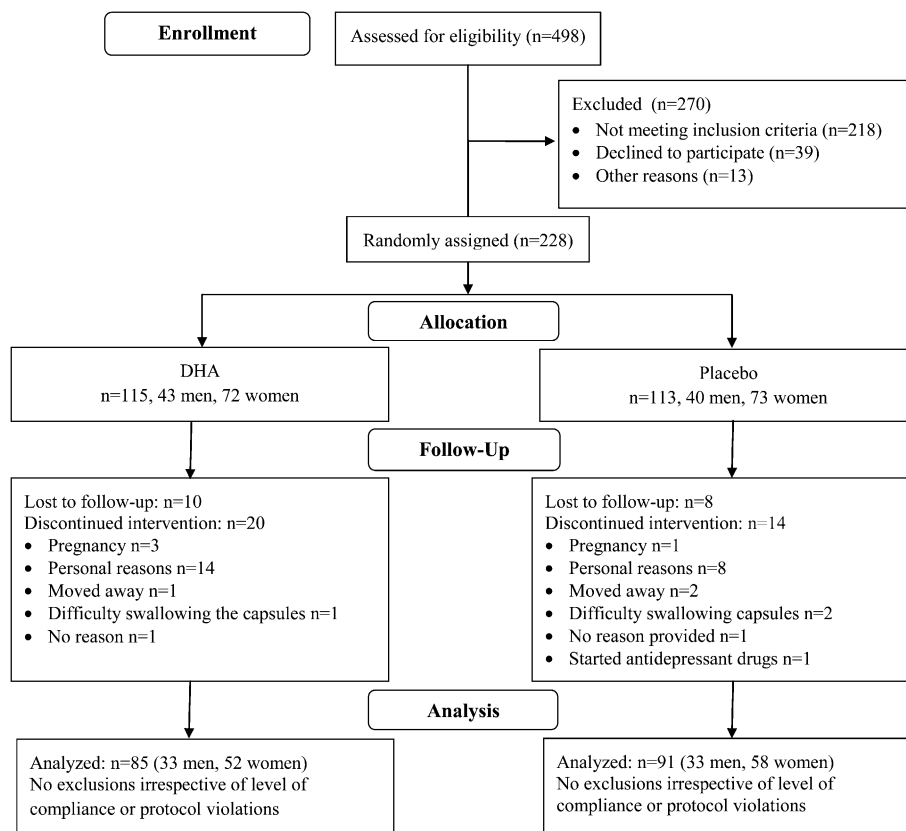
$$\text{Processing speed} = z_{\text{finding As}} \quad (7)$$

Statistical analyses were performed with IBM SPSS statistics software (version 20; IBM Corp).

## RESULTS

The flow of participants through the study is summarized in **Figure 1**. Of 228 participants who were randomly assigned to treatments, 52 subjects were lost to follow-up or discontinued the intervention for various reasons ( $n = 30$  in the DHA group;  $n = 22$  in the placebo group) (Figure 1). The final analysis was conducted in 176 participants [ $n = 85$  in the DHA group (33 men and 52 women);  $n = 91$  in the placebo group (33 men and 58 women)] for whom baseline and end data were available irrespective of the level of compliance or protocol violations.

Baseline characteristics of subjects are summarized in **Table 2**. Participants were mostly European, had English as their first language, and were highly educated (most having tertiary qualifications). DHA and placebo groups did not differ with regard to baseline characteristics (Table 2) or cognitive tests



**FIGURE 1.** Participant flow through the study.



**TABLE 2**

Baseline characteristics by randomly assigned treatment groups

Variables	DHA (n = 115)	Placebo (n = 113)	P <sup>1</sup>
M/F (n)	43/72	40/73	0.75
Age (y)	33.4 ± 7.76 <sup>2</sup>	33.2 ± 7.90	0.82
Ethnicity (%)			NA <sup>3</sup>
European	78.2	80.9	
Maori/Pacific	5.5	0	
Asian	14.5	13.6	
Other	1.8	5.5	
English as first language (%)	86	81	0.27
Education (%)			NA <sup>3</sup>
No qualifications	0	1	
Secondary school	30	26	
Tertiary	70	74	
BMI (kg/m <sup>2</sup> )	25.7 ± 4.89	24.9 ± 4.63	0.21
APOE4 carriers (n = 176) [n (%)] <sup>4</sup>	29 (34)	25 (28)	0.41

<sup>1</sup> Values were derived by using an independent *t* or chi-square test (2 tailed).

<sup>2</sup> Mean ± SD (all such values).

<sup>3</sup> NA, chi-square test was not appropriate; >20% cells have expected counts <5.

<sup>4</sup> APOE analyses were available only for participants who completed the 6-mo intervention.

scores (see Table 1 under “Supplemental data” in the online issue). A significant sex × treatment interaction was seen for the first language. The DHA group contained a greater proportion of men whose first language was English than did the placebo group (88% compared with 68%, respectively; *P* = 0.02). However, all participants had a good knowledge of English. Of the 176 participants who completed the study, 31% of subjects were carriers of the APOE4 allele (43 E3/E4, 5 E4/E4, and 6 E2/E4) and 69% of subjects were noncarriers (103 E3/E3 and 19 E2/E3). At baseline, women scored higher than men did on the

following cognitive tasks: delayed picture recognition [mean difference (95% CI): 1.23% (0.04%, 2.42%); *P* = 0.04], delayed word recognition [2.56% (0.01%, 5.11%); *P* = 0.049], finding As task [6.58 (3.23, 9.94); *P* < 0.001], and delayed word recall [3.97% (0.33%, 7.61%); *P* = 0.03] (data not shown). No significant differences were seen between men and women for any other cognitive tasks (data not shown).

Dropouts did not differ from the participants who completed the study for any baseline characteristics (data not shown) except for age. Dropouts were 2.76 y (95% CI: 0.35, 5.18 y) younger than participants who completed the study (mean ± SD: 31.2 ± 7.97 compared with 33.9 ± 7.7 y, respectively; *P* = 0.03).

The median (25, 75 percentile) consumption of seafood over the duration of the trial was 1.08 portions/wk (0.43, 2.15 portions/wk), of which only 0.01 portions/wk (0, 0.07 portions/wk) were from fatty seafood sources. Treatment groups did not differ with regard to seafood intake (*P* = 0.44).

Compliance rates were 87.0 ± 14.3% compared with 88.8 ± 13.3% in DHA and placebo groups, respectively, and did not differ significantly. A significant increase in erythrocyte DHA levels compared with the placebo of 2.7% (95% CI: 2.37%, 3.03%) of total fatty acids confirmed compliance to the DHA treatment (Table 3). Erythrocyte EPA and the n-3 index increased, whereas docosapentaenoic acid, arachidonic acid (AA), AA:EPA ratio, and AA:DHA ratio decreased significantly with DHA compared with placebo treatment (*P* < 0.001). Neither men compared with women nor APOE4 carriers compared with noncarriers differed with regard to baseline erythrocyte fatty acid levels or changes in erythrocyte fatty acid levels in response to treatments (data not shown).

Effects of treatment on the cognitive-function domains are summarized in Table 4. See Table 2 under “Supplemental data” in the online issue for a summary of the results of individual cognitive-function tests. A significant effect of sex × treatment interaction (*P* = 0.01) was seen for episodic memory. Although no effects were seen in men, episodic memory improved

**TABLE 3**

Changes within and between treatments in erythrocyte arachidonic acid, long-chain omega-3 fatty acids, and omega-3 index from baseline to 6 mo<sup>1</sup>

Variables	DHA (n = 83)			Placebo (n = 90)			DHA compared with placebo	
	Baseline	6 mo	Change <sup>2</sup>	Baseline	6 mo	Change	Difference <sup>3</sup>	<i>P</i>
AA (percentage of total fatty acids)	11.2 ± 1.63 <sup>4</sup>	10.4 ± 1.45	-0.88 ± 1.15	11.2 ± 1.70	11.2 ± 1.73	0.01 ± 1.29	-0.89 (-1.26, -0.52)	<0.001
EPA (percentage of total fatty acids)	0.61 ± 0.34	0.81 ± 0.41	0.20 ± 0.22	0.54 ± 0.31	0.52 ± 0.31	-0.01 ± 0.25	0.21 (0.14, 0.28)	<0.001
DPA (percentage of total fatty acids)	2.81 ± 0.77	2.48 ± 0.85	-0.33 ± 0.73	2.71 ± 0.81	2.70 ± 0.88	-0.01 ± 0.60	-0.32 (-0.52, -0.12)	0.002
DHA (percentage of total fatty acids)	5.28 ± 1.35	7.91 ± 1.65	2.62 ± 1.27	5.06 ± 1.76	4.98 ± 1.60	-0.08 ± 0.88	2.70 (2.37, 3.03)	<0.001
AA:EPA	23.1 ± 12.9	15.2 ± 6.38	-7.84 ± 11.6	23.3 ± 9.59	23.8 ± 9.92	0.53 ± 9.62	-8.37 (-5.06, -11.7)	<0.001
AA:DHA	2.30 ± 0.84	1.37 ± 0.38	-0.93 ± 0.62	2.51 ± 1.0	2.53 ± 1.02	0.02 ± 0.45	-0.95 (-1.11, -0.79)	<0.001
Omega-3 index (%)	5.89 ± 1.42	8.72 ± 1.72	2.82 ± 1.34	5.59 ± 1.90	5.50 ± 1.72	-0.09 ± 0.98	2.92 (2.56, 3.27)	<0.001

<sup>1</sup> Includes only participants with erythrocyte fatty acid analysis at baseline and 6 mo. Men and women did not differ, and therefore, only results of total treatment groups are presented. *P* values were derived by using independent *t* test (2 tailed). AA, arachidonic acid (20:4n-6); DHA, 22:6n-3; DPA, docosapentaenoic acid (22:5n-3); EPA, 20:5n-3; omega-3 index, percentage of EPA + DHA of total erythrocyte fatty acids.

<sup>2</sup> Baseline and 6-mo values differed significantly within treatment (*P* < 0.001; dependent *t* test, 2 tailed).

<sup>3</sup> All values are means; 95% CIs in parentheses.

<sup>4</sup> Mean ± SD (all such values).



**TABLE 4**  
Changes within and between treatments in cognitive-function test z scores from baseline to 6 mo<sup>1</sup>

Variables	DHA ( <i>n</i> = 85)				Placebo ( <i>n</i> = 91)				DHA compared with placebo			
	Baseline	6 mo	Change	Baseline	6 mo	Change	Difference	<i>P</i> -treatment effect	<i>P</i> -sex × treatment	<i>P</i> - <i>APOE</i> × treatment		
<b>Episodic memory</b>												
Total	-0.06 ± 0.76 <sup>2</sup>	0.19 ± 0.63	0.26 (0.14, 0.38) <sup>3,4</sup>	-0.14 ± 0.71	0.07 ± 0.68	0.21 (0.09, 0.32) <sup>4</sup>	0.05 (-0.11, 0.22)	0.52	0.01	0.39		
Men	-0.21 ± 0.82	-0.002 ± 0.74	0.15 (-0.04, 0.34)	-0.23 ± 0.73	0.18 ± 0.66	0.32 (0.14, 0.50) <sup>4</sup>	-0.17 (-0.43, 0.09)	0.20	—	—		
Women	0.04 ± 0.71	0.31 ± 0.52	0.37 (0.23, 0.51) <sup>4</sup>	-0.09 ± 0.70	0.01 ± 0.69	0.09 (-0.05, 0.23)	0.28 (0.08, 0.48)	0.01	—	—		
<b>RT<sup>5</sup> of episodic memory</b>												
Total	0.04 ± 0.95	-0.19 ± 0.72	-0.28 (-0.39, -0.17) <sup>4</sup>	0.14 ± 0.94	0.01 ± 0.89	-0.10 (-0.20, 0.01) <sup>4</sup>	-0.18 (-0.33, -0.03)	0.02	0.81	0.87		
<b>Working memory</b>												
Total	-0.07 ± 0.76	0.15 ± 0.62	0.22 (0.11, 0.32) <sup>4</sup>	-0.09 ± 0.74	0.01 ± 0.79	0.12 (0.01, 0.22) <sup>4</sup>	0.10 (-0.04, 0.25)	0.17	0.37	0.46		
<b>RT of working memory</b>												
Total	0.10 ± 1.01	-0.33 ± 0.82	-0.51 (-0.66, -0.35) <sup>4</sup>	0.20 ± 1.09	0.01 ± 0.99	-0.15 (-0.30, 0.00) <sup>4</sup>	-0.36 (-0.58, -0.14)	0.002	0.03	0.78		
Men	0.06 ± 1.03	-0.50 ± 0.74	-0.66 (-0.90, -0.41) <sup>4</sup>	0.16 ± 1.07	0.11 ± 1.02	-0.06 (-0.31, -0.19)	-0.60 (-0.95, -0.25)	0.001	—	—		
Women	0.31 ± 1.01	-0.22 ± 0.85	-0.36 (-0.55, -0.17) <sup>4</sup>	0.23 ± 1.11	-0.05 ± 0.98	-0.24 (-0.43, -0.06) <sup>4</sup>	-0.12 (-0.38, 0.15)	0.39	—	—		
<b>Attention</b>												
Total	0.06 ± 0.57	0.06 ± 0.55	0.06 (-0.08, 0.20)	-0.06 ± 0.62	-0.02 ± 0.70	-0.04 (-0.18, 0.10)	0.10 (-0.10, 0.30)	0.32	0.14	0.51		
<b>RT of attention</b>												
Total	-0.09 ± 0.75	-0.03 ± 0.76	0.07 (-0.04, 0.17)	0.00 ± 0.77	0.01 ± 0.74	0.02 (-0.08, 0.13)	0.04 (-0.11, 0.19)	0.59	0.22	0.80		
<b>Processing speed</b>												
Total	-0.11 ± 1.03	0.19 ± 1.15	0.27 (0.14, 0.40) <sup>4</sup>	-0.23 ± 0.83	0.16 ± 0.93	0.38 (0.25, 0.50) <sup>4</sup>	-0.11 (-0.29, 0.07)	0.24	0.13	0.65		

<sup>1</sup> Includes only participants with cognitive-function scores at baseline and 6 mo. The DHA group included 33 men and 52 women. The placebo group included 33 men and 58 women. A decrease in the reaction time of cognitive tasks indicates an improvement. *P* values were derived by using ANCOVA (adjusted for baseline cognitive function score, baseline DHA concentrations, first language, age, and education). Sex and *APOE* were added as independent variables to test for sex × treatment and *APOE* × treatment interactions.

<sup>2</sup> Mean ± SD (all such values).

<sup>3</sup> Mean; 95% CI in parentheses (all such values). All values were adjusted for baseline cognitive function z score, baseline DHA concentrations, first language, age, and education.

<sup>4</sup> Baseline and 6-mo z scores differed significantly within treatment (*P* < 0.05; dependent *t* test, 2 tailed).

<sup>5</sup> RT, reaction time.

significantly (by 0.28 SDs) in women who consumed DHA compared with women who consumed the placebo. This improvement equated to correctly remembering or recognizing approximately one more word or picture compared with the placebo group.

The RT of episodic memory improved significantly after DHA treatment irrespective of sex, and the DHA group responded faster (by 0.18 SDs) to these tasks than the placebo group did.

DHA did not significantly affect the working memory domain, but the RT of working memory improved significantly (by 0.36 SDs) in the DHA group compared with the placebo group. A significant sex  $\times$  treatment interaction was also seen ( $P = 0.03$ ). Although RTs improved numerically for both sexes after DHA compared with placebo treatment, this improvement was only significant in men (by 0.6 SDs). This result equated to men in the DHA group having completed the working memory task 223 ms ( $-354$ ,  $-92.4$  ms) (20%) faster than did men in the placebo group.

No significant effects of DHA were seen on attention or the RT of attention. However, there was limited room for improvement on the accuracy of attention tasks with baseline scores on attention tasks  $>96\%$  (CRT  $>97\%$ , digit vigilance  $>96\%$ , and Stroop test  $>97\%$ ) (see Table 2 under "Supplemental data" in the online issue).

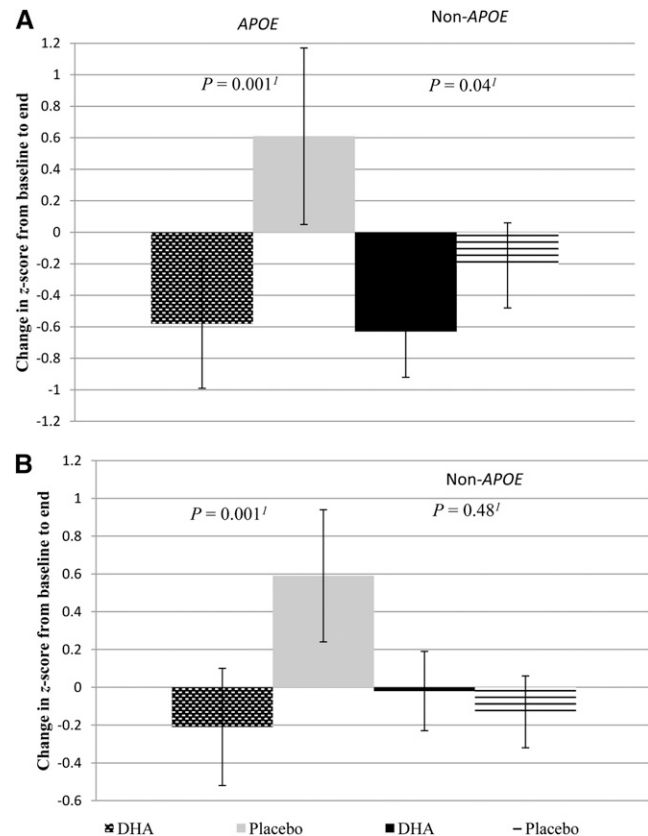
The processing speed did not differ significantly between DHA and placebo groups.

*APOE4* carriers and noncarriers did not differ at baseline with regard to cognitive domains. *APOE* status did not affect treatment responses on any cognitive domains (Table 4). Because no effects of *APOE*  $\times$  treatment interaction were observed, results stratified by *APOE* groups are not presented. *APOE*  $\times$  sex  $\times$  treatment interactions were observed for RTs of working memory ( $P = 0.02$ ) and attention ( $P = 0.005$ ). Both male *APOE4* carriers and noncarriers who received DHA showed improvements in the RT of working memory compared with those in the placebo group, but the effect was considerably greater in *APOE4* carriers [mean (95% CI) differences between treatments were  $-1.19$  SD ( $-1.89$ ,  $-0.50$  SD) ( $P = 0.001$ ) in *APOE4* carriers and  $-0.42$  SD ( $-0.81$ ,  $-0.02$  SD) ( $P = 0.04$ ) in *APOE4* noncarriers] (Figure 2A). The large effect in *APOE4* carriers was due to both improvements in the DHA group and a worsening of performance in the placebo group. A similar effect was seen in male *APOE4* carriers for RTs of attention (Figure 2B) with an improvement in the DHA group and worsening in the placebo group, which resulted in a significant difference between groups [mean (95% CI):  $-0.80$  SD ( $-1.27$ ,  $-0.33$  SD) ( $P = 0.005$ ). The RT of attention was not affected in *APOE4* noncarriers.

With regard to side effects, a significantly greater proportion of participants in the DHA-treatment group reported burping and unpleasant breath [burping: 49% compared with 22%, respectively ( $P < 0.001$ ); unpleasant breath: 39% compared with 18%, respectively ( $P = 0.001$ ); chi-square test], but the effects were rated as minor (1 and 2 on a scale from 1 to 10).

## DISCUSSION

This study showed, for the first time to our knowledge, that DHA supplementation improved memory and RTs of memory in healthy young adults whose habitual diet was low in DHA, and sex modulated the response. DHA supplementation significantly



**FIGURE 2.** Adjusted mean (95% CI) changes in z scores from baseline to end in RT for working memory (A) and attention (B) in male *APOE4* carriers compared with noncarriers who consumed DHA compared with a placebo (*APOE4*: DHA,  $n = 11$ ; placebo,  $n = 7$ ; *APOE4* noncarriers: DHA,  $n = 22$ ; placebo,  $n = 26$ ). A decrease in RT indicates an improvement. <sup>†</sup>*P* values indicate differences between DHA and placebo groups within *APOE* groups and were derived by using ANCOVA (adjusted for baseline RT, baseline DHA concentrations, first language, age, and education). *APOE*  $\times$  sex  $\times$  treatment interaction: RT for working memory,  $P = 0.02$ ; RT for attention,  $P = 0.005$ . RT, reaction time.

improved the RT of episodic memory, whereas the accuracy of episodic memory was improved in women, and the RT of working memory was improved in men. *APOE* did not have an effect on changes in cognitive function in response to DHA supplementation, but there was some indication that, when stratified by sex, *APOE* may have modulated effects on RTs of working memory and attention. Because the study did not have sufficient statistical power to investigate this interaction, these effects are only preliminary. No conclusions could be drawn regarding the effect of DHA on the accuracy of attention because of ceiling effects in test performance. The processing speed was not affected by DHA supplementation.

The few RCTs undertaken in healthy young adults have failed to show any effects of LC n-3 PUFAs on episodic or working memory (12, 14–16), RTs of memory (14, 15), attention (14, 15), or processing speed (16). However, none of these studies examined sex interactions. The failure to examine sex interactions may have led to inaccurate conclusions because if a sex dimorphism exists, the combination of sexes may have cancelled out or dilute any potential effects. Some studies used small dosages (12, 15) or had small sample sizes (12–14), and all studies were of relatively short duration, with a range from 4



to 12 wk (12–16). A duration >12 wk and, on the basis of the current study,  $\geq 6$  mo may be needed to achieve measurable effects on cognitive function in adults. All of these limitations were overcome in the current study; the intervention period was of adequate duration (6 mo), a relatively large DHA dosage was used, although such a level can be achieved by dietary changes, and the study had sufficient statistical power to investigate interaction effects of sex and *APOE*. The comprehensive battery of cognitive tests have previously been shown to be sensitive to nutrition interventions and were performed under rigorous standardized conditions that reduced variation because of environmental factors (48). A limitation was the lack of statistical power to investigate *APOE* interactions stratified by sex.

The increased consumption of DHA in the current study resulted in an increase of 2.6% of total fatty acids in erythrocyte DHA levels and also lowered AA:DHA and AA:EPA ratios, which may have contributed to the cognitive improvements shown in the study by altering membrane fluidity and decreased production of proinflammatory eicosanoids (1, 3). DHA is likely to affect brain function by several possible mechanisms as previously reviewed (1–3). Its incorporation into the neuronal membrane lipid bilayer is essential for neuroplasticity, the promotion of neurogenesis, neurite outgrowth and synaptogenesis, and the maintenance of membrane fluidity and membrane protein function, which, in turn, affects the speed of signal transduction, neurotransmission (1–3), and regulation of brain glucose uptake (49). DHA has also been shown to reduce the vascular tone, which is likely to increase cerebral blood flow during cognitive tasks (14). Unesterified DHA, which is released from the *sn*2 position of phosphoglycerides by phospholipase A2 is likely to influence inflammation via a variety of mechanisms, including the downregulation of inflammatory cytokine production and by acting as a precursor of the docosanoid family of compounds (neuroprotectins and resolvins) that resolve neuroinflammation and inhibit oxidative stress-induced neuronal apoptosis (1, 3).

Memory domains were affected differently by DHA supplementation in men and women. In women, episodic memory improved, whereas in men, RTs of working memory improved compared with in the placebo group. Sex differences in cognitive test performance may be explained by the use of different problem-solving strategies by men and women as indicated by differences in the functional organization of the brain when performing memory tasks (19, 20).

The *APOE4* allele, which was carried by 31% of participants in the current study, is the major common genetic risk factor for Alzheimer's disease (21, 22) with an  $\sim 3$ - and 15-fold increase in risk in *APOE3/E4* and *APOE4/E4* individuals relative to the wild-type genotype (21). Although the physiologic basis is unknown, there have been reports that the cognitive response to LC n-3 PUFAs may be *APOE* dependent. Improvements in attention in >65-y-olds were seen only in *APOE4* carriers (10), whereas Quinn et al (32) showed improvements in cognitive function only in *APOE4* noncarriers with Alzheimer's disease. Evidence for the role of *APOE4* in the young adult brain is still emerging, but structural and functional neurologic changes are seen in *APOE4* carriers decades before the appearance of any cognitive or clinical symptoms (27–30). *APOE4* in young adults is associated with abnormally low rates of glucose metabolism that occurs as early as in 20–39-y-olds (30) and re-

gional brain atrophy (50, 51). Surprisingly, young (20–35-y-old) *APOE4* carriers have been shown to perform better on cognitive tasks than noncarriers have (25, 26) and showed increased brain activation in the frontal and temporal regions during memory tasks (28).

To our knowledge, the current study is the first to examine the cognitive response to DHA supplementation in younger adults according to *APOE*. We saw no evidence for a differential cognitive performance with DHA treatment. Although no effect of the *APOE4* allele carrier status was observed for the responses in the group as a whole, the data were strongly suggestive of sex dimorphisms. There was some indication that, when stratified by sex, improvements in RTs of working memory and attention with DHA compared with placebo were more pronounced in male *APOE4* carriers than in noncarriers. The effects were due to both improved scores in the DHA group and worsening scores in the placebo group. This novel finding of a potentially greater effect of DHA in RTs of memory and attention in male *APOE4* carriers requires additional investigation, and the biological basis for the interaction needs to be investigated.

More-robust RCTs that are long enough ( $\geq 6$  mo) and take into account the habitual intake of LC n-3 PUFAs are needed to confirm the findings of this study and to determine the most effective dosage of DHA for optimal cognitive function. It is important that any future trials of DHA on cognitive function stratify for sex, and this should be planned into the study design from the start so that sex-stratified random assignment and recruitment of sufficient numbers of men and women is ensured. Future trials that investigate the effects of *APOE* should ensure a sufficient statistical power to investigate the effects of *APOE* stratified by sex.

In conclusion, DHA supplementation improved memory and RTs of memory in healthy young adults whose habitual diet was low in DHA and sex modulated the response to DHA supplementation. *APOE* did not modulate the response to treatment in the group as a whole, but there was some indication that, when stratified by sex, *APOE* may have resulted in greater improvements in RTs in male *APOE4* carriers, but this needs to be confirmed in future studies. These memory-related cognitive domains are the building blocks of more-complex cognitive functions or behaviors that are common in everyday life (20, 48). Thus, young healthy adults may cognitively benefit from an increased consumption of DHA.

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The authors' responsibilities were as follows—WS: was the main author of the manuscript, designed the research (project conception, development of the overall research plan, and study oversight), conducted the research (hands-on conduct of the research and data collection), performed statistical analyses, and had primary responsibility for the final content of the manuscript; CAC: designed the research (project conception, development of the overall research plan, and study oversight), conducted the research (hands-on conduct of the research and data collection), analyzed cognitive data, critically revised the manuscript, and approved of the final version of the manuscript; JP and SRH: designed the research (project conception and development of the overall research plan), critically revised the manuscript, and approved the final version of the manuscript; AMM: provided significant advice regarding all aspects related to *APOE* analysis, interpretation of *APOE* data, and contribution to the writing up of these aspects in the manuscript, critically revised the manuscript, and approved the final version of the manuscript; CH: designed the computerized cognitive test battery and provided advice regarding the analysis of cognitive data, critically revised



the manuscript, and approved of the final version of the manuscript; and DK: designed the computerized cognitive test battery, critically revised the manuscript, and approved of the final version of the manuscript. DK has previously received funding from Efamol, Martek, and Ginsana SA for DHA research. WS, CAC, JP, SRH, AMM, and CH had no conflicts of interest.

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